Dec 23 192

VOLUME 12 NUMBER 11

NOVEMBER, 1922

Phytopathology

Official Organ of the

American Phytopathological Society

CONTENTS

The bacterial spot of pepper ..... B. B. Higgins 601

A new host for the fire blight organism, Bacillus amylovorus ..... Laetitia M. Snow 617

Yeast-spot of Lima beans ..... S. A. Wingard 625

Phytopathological notes 633

PUBLISHED MONTHLY BY THE SOCIETY

OFFICE OF PUBLICATION

8 West King Street, Lancaster, Pa.

Entered as second-class matter at the post office at Lancaster, Pa.

Price { $5.00 per year, United States and dependencies, Mexico and Cuba
$5.25 per year, Canada
$5.50 per year, elsewhere

DIFCO

DEHYDRATED CULTURE MEDIA

for the

PLANT PATHOLOGIST and MYCOLOGIST

BACTO-CORN MEAL AGAR DEHYDRATED

The water soluble ingredients of CORN MEAL combined with AGAR

powder makes more than 50 liters of medium.

BACTO-PRUNE AGAR DEHYDRATED

A clarified infusion of the best grades of PRUNES combined with BAC

pound makes more than 15 liters of medium.

PACKED IN QUARTER AND ONE POUND BOTTLES, IN POWDER

Specify "DIFCO"

The Trade Name of the Pioneer in the Research and Development of Bacto

Dehydrated Culture Media

DIGESTIVE FERMENTS COMPANY

DETROIT, MICHIGAN, U. S. A.

An Opinion of Smith's Bacterial Diseases of Plants

"It is a valuable stimulus to a correct scientific attitude and many an investigator would do well in the rush of work to sit down for an hour and peruse Part V of this book."—*The Philippine Journal of Science.*

By Erwin F. Smith, in charge of Laboratory of Plant Pathology, Bureau of Plant Industry, Washington, D. C. Octavo, of 688 pages, illustrated. Cloth, $10.00 net.

W. B. SAUNDERS COMPANY

PHILADELPHIA LONDON

The Surest and Purest For Dusting and [illegible]

For dusting and [illegible] the "ANCHOR" BRAND SUBLIMED VELVET FLOWERS and the "EAGLE" BRAND SUBLIMED FLOWERS OF SULPHUR are superior and will give the surest and quickest results. These SNOW FLAKE-LIKE particles float to every surface and crevice of the plant.

They are the highest grades of sublimed sulphur produced. Do not adulterate these sulphurs with Lime, Kaolin, or any other inert material. Coating the sulphur with any such material PREVENTS the FUMIGATING ACTION caused by the sun's rays.

For increasing crop yields, control of potato scab, sugar beet scab, sweet potato scruff, onion thrip, etc., and for repelling cut-worms, wire-worms, etc., which cause root knot and other root diseases, use "ORO" [illegible] SPECIALLY PREPARED AGRICULTURAL COMPOUNDS.

Send for circulars Nos. 6, 10 and 15, regarding dusting and agricultural compounds.

Stauffer Chemical Company of Texas

711 Scanlan Building

Houston Texas

PHYTOPATHOLOGY

VOLUME XII NUMBER 11

NOVEMBER, 1922

THE BACTERIAL SPOT OF PEPPER[1]

B. B. HIGGINS

WITH PLATES XXXI AND XXXII AND FIVE FIGURES IN THE TEXT

A bacterial spot or wart on the leaves, stems, and fruits of peppers (*Capsicum annuum* L.) has been prevalent and quite destructive in the Pimento growing region about Griffin, Georgia, during the last three seasons. It was first brought to my attention during the spring of 1920, when some 2000 Pimento pepper plants were bought from a local grower, for field work in relation to a study of pepper fruit rots. The plants were examined carefully for signs of disease before being set in the field. A few were thrown out because of small brownish spots on the leaves. These spots were distinctly different from the common Cercospora leaf spot, especially in the absence of the white center so characteristic of the Cercospora spot.

The remainder of the plants were set in the field about the middle of May, together with several rows of other healthy plants. Six weeks later every plant in the lot purchased from the local grower was diseased and the disease had spread to most of the other plants in the field. Many of the diseased leaves had already dropped off. During August many of the plants were almost completely defoliated.

SYMPTOMS

The spots on the leaves, generally, first appear as small, circular, pale green pimples. They are raised on the under surface of the leaf and occasionally on both surfaces. Usually there is a slight depression or concavity on the upper surface. On old leaves the first noticeable sign of infection is often a darker green, water-soaked spot. The behavior and final appearance of the spots vary considerably with the age and growth conditions of the leaves attacked. Usually the center of the spot dies and collapses after a few days. The spot continues to enlarge

[1] Paper Number 17, Journal Series, Georgia Agricultural Experiment Station.

for some time, forming a circular or oblong pale-yellow or straw-colored spot 1 to 10 mm. in diameter with a border of water-soaked tissue which finally turns dark brown. On old leaves, late in the fall, the spots are dark brown with a paler brown center on the lower surface. Occasionally two or more spots coalesce and form a larger spot of a more irregular outline. However, large areas of the leaf are never killed. Usually, when infection is abundant, the spots are small and do not coalesce, the entire leaf turning yellow and dropping off, or quite as often dropping off while still green.

On the stems the disease is not very noticeable. Small, elongated, raised cankers 3 to 5 by 1 to 2 mm. in diameter appear and go through much the same series of transitions as the spots on the leaves. The center of the cankers finally becomes roughened and light brown in color; but the spots do not collapse as they do on the leaves.

On the fruits the spots are much more conspicuous. They are circular in outline, 2 to 5 mm. in diameter, raised with a cracked and roughened surface, (Pl. XXXII) giving them a wart-like appearance. They are at first pale green but soon turn brown. During damp weather decay organisms often enter through these spots and cause the collapse of the surrounding tissue and finally of the entire fruit.

CAUSE OF THE DISEASE

Soon after the disease was noticed in the fields, isolations were made by dilution cultures on agar plates. The leaves were washed, immersed for one minute in a 1–1000 mercuric chloride solution, and then washed in sterile water. The diseased spots were then removed with flamed tweezers and crushed in tubes of melted agar from which other dilution poured plates were made. When young spots were washed and cultured in this manner pure cultures of a yellow-pigment-forming bacterium, which reproduced the disease when sprayed onto pepper plants, were obtained.

MORPHOLOGY OF THE ORGANISM

Vegetative cells. These bacteria were found to be short rods with rounded ends. When grown on beef extract agar and stained with gentian violet or methylene blue the cells measured 1 to 1.8 by 0.5 to .7 μ. They usually occur singly, but occasionally in pairs and rarely in short chains. The nature of the substratum seemed to affect the size and shape of the bacterial cells only slightly. In carbohydrate media there seemed to be a slightly increased tendency to form chains.

Capsules. Capsules are present and quite distinct on certain media. They have been demonstrated in 24 hour old beef extract agar cultures,

but they are very much more distinct in old cultures on sweet potato plugs (Fig. 1).

Endospores. No evidence of endospores could be obtained by staining. Temperature reactions of old cultures indicate that they are not formed.

Motility. The organism is not very actively motile. Hanging drop mounts from more than a dozen young cultures were examined before motility was satisfactorily demonstrated. Usually only a very small percentage of the cells are motile, though occasionally a larger number

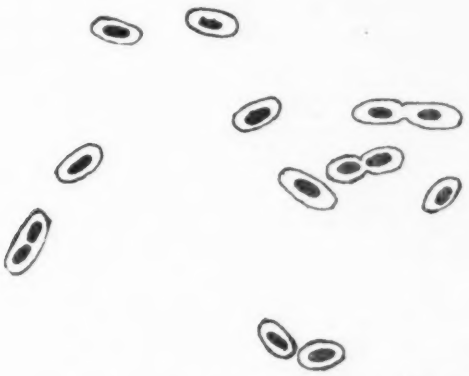


Fig. 1. Rods with capsules from a 20-day old sweet potato culture, stained by the Welch method. × 3700.

Fig. 2 Monoflagellate rods from a 20-hour old agar culture stained by Loeffler's method. × 3200.

were found active. Flagella stains demonstrated the same thing. That is, flagella could be found on only a few cells; although they stained readily with Loeffler's methylene blue or with carbol-fuchsin when preceded by a mordant. It has only a single polar flagellum which is usually about two and a half times the length of the cell (Fig. 2).

Involution Forms. No involution forms of any kind were found. In staining for capsules by the Welch method many irregular forms were found, but these were evidently due to plasmolysis from the salt solution

used in washing. When mounts from the same cultures were stained with gentian violet or with methylene blue the cells appeared entirely normal.

Staining. The organism takes the routine bacterilogical stains readily; but it is Gram negative. Mounts from eighteen and twenty-four hour beef extract agar cultures were tested on several occasions, but with Gram's stain the results were always negative.

CULTURAL CHARACTERISTICS

All cultural and physiological studies were made with at least four isolations of the organism as follows: 1, from a leaf spot in 1920; 2, from a leaf spot in 1921; 3, from a fruit spot; and 4, from the hypocotyl of a young seedling. In addition, the reaction of several other isolations were from time to time compared with these four.

The organism is a moderately rapid grower. On poured plates of beef extract agar incubated at 25° C. the colonies are visible to the unaided eye after about 40 hours. In plate cultures it is easily separated from the saprophytic forms, commonly occurring in or on the diseased spots of the host plant, by means of the color and general appearance of the colonies. By reflected light the surface colonies are some shade of lemon yellow from the time they appear until they dry up. They are wet, shiny, and slightly convex. By transmitted light they are translucent, slightly opalescent, and the larger colonies show a distinctly translucent border around the more opaque center. Until about the fifth day the edges of the colonies are smooth. In older plates they become reticulate or lobate (Pl. XXXI, fig. 1). The submerged colonies are lens shaped, but often break through and spread out on the surface. In old cultures they usually become more or less irregularly lobed (Pl. XXXI, fig. 2). The colonies between the agar and the glass plate are thin, delicate, and of a pale cream yellow color.

The growth in agar stroke cultures is filiform at first but after a few days it becomes more or less echinulate. In agar stab cultures the surface growth is not abundant, not covering the surface of the agar. The submerged growth is echinate.

Gelatin. On beef extract gelatin the color of the bacterial growth is approximately the same as that produced on agar. In thinly sown plates the colonies are 1 to 1.5 cm. in diameter, after 10 day incubation at 18° C., with a cup-shaped liquefied area underneath. In stab cultures some growth occurs all along the stab, although no liquefaction is discernible except along the upper portion. In stab cultures in freshly prepared gelatin liquefaction is evident at the upper end of the stab after 48-hours growth at 20° C. A cup shaped or turnip-shaped area

0.5 cm. deep is liquefied. By the fourth day this area has become more crateriform. By the seventh day the entire upper portion of the gelatin is liquefied to the depth of 1 cm. or more, and below this a flat Lima bean-shaped area is liquefied with the bacteria settled into a sickle-shaped mass at the bottom. In older gelatin which has been heated several times the liquefaction is more rapid but much less characteristic.

Bouillon. In beef extract broth the growth seems to occur almost entirely on the surface, and, if allowed to stand undisturbed, the liquid is not very decidedly clouded. When shaken, the growth becomes distributed, producing a decided cloudiness, but gradually settles to the bottom. In cultures 3 weeks or more old the liquid is clear and more translucent than sterile broth, the bacterial growth having formed a viscid mass at the bottom. The entire liquid gradually becomes lemon yellow and mucilaginous. The bacterial growth at the bottom is straw yellow and has about the consistency of egg albumen.

Potato. On steamed Irish potato plugs growth was a little more abundant than on beef extract agar, slightly viscid, of a dense cream yellow color, but lacking the opalescence of agar cultures. Where the plugs were very moist the growth was of a more fluid nature, and had a tendency to spread and flow to the bottom of the tube. There was very little digestion of the potato tissue.

Sweet potato. On steamed sweet potato plugs growth is very rapid and abundant, decidedly more abundant than on any other vegetable medum tried. By the end of 10 days at a temperature of 20° to 25° C. the plugs are almost engulfed in a yellow, viscid mass which flows to the bottom of the tube. By the end of a month the plug is almost entirely digested. Usually a bluish-green color develops in the sweet potato tissue by the fifth day. This color did not develop in plugs from sweet potatoes taken from a curing house late in the spring. This variation was probably due to changes in the latex of the potatoes.

Pepper fruit agar. Pepper fruit agar was prepared in two ways. By the first method 400 grams of green Pimento pods were minced fine and boiled for an hour in a small amount of tap water. The liquid was then drained off, filtered, and made up to 1500 cc. To this liquid 1.5 per cent agar was added, steamed until dissolved, filtered, tubed, and sterilized. By the second method only the sap of the pepper was used. The Pimento pods (400 grams) were ground in a food chopper and the sap (300 cc.) pressed out, filtered, and then made up to 1500 cc. by the addition of tap water. Agar was added as above.

The freshly expressed sap had a hydrogen-ion concentration of approximately pH 5.2. The acidity was slightly increased by sterili-

zation. The agar prepared by the first method was a little more acid, having a pH value of 5.0.

The sap made much the clearer, nicer agar. When slants of either were heavily inoculated, some growth was produced, but when a small amount of inoculum was employed no growth resulted. However, after neutralization by the addition of a normal sodium hydroxide solution, growth was very good—much more abundant than on beef extract agar.

Egg albumen. Good growth was produced in a 2 per cent solution of commercial dried egg albumen, with or without the addition of 0.5 gram of KH_2PO_4 per liter.

Asparagin. No growth was produced in asparagin solution.

Media lacking organic nitrogen. No growth was produced in a solution of inorganic salts plus saccharose (K_2HPO_4, 0.5 gram; $CaCl_2$, 0.5 gram; KNO_3, 1.0 gram; saccharose, 10.0 grams; and water, 1000.0 cc.). It seemed that some organic nitrogen was required, though the amount needed was not large. Good growth was obtained on a 0.1 per cent solution of peptone, especially when dextrose or saccharose was added. Increasing the proteid content to the amount recommended by the Committee (1) on the Descriptive Chart of the Society of American Bacteriologists by the addition of beef extract and peptone (0.3 per cent beef extract and 0.5 per cent peptone) did not noticeably increase the growth.

Carbohydrate media. The organism responds very quickly to the addition of dextrose or saccharose to beef extract agar or broth. On stab cultures in tubes of beef extract agar the growth never covers the surface of the agar. When 3 per cent of either of the above sugars are added a solid mass of bacterial growth 1 to 1.5 cm. deep is produced above the agar. Lactose, maltose, mannite, glycerine, or starch did not increase the growth by any such appreciable amount, although some of them, at least lactose and starch, are used to some extent.

PHYSIOLOGY

Liquefaction of gelatin. In gelatin stab cultures incubated at 18° to 20° C. liquefaction begins on the second day and proceeds slowly until growth ceases which is about the end of the sixth week. If the gelatin is more than two inches deep in the tube, it is usually not all liquefied.

Relation to free oxygen. The organism is a strict aerobe. No growth is produced in the closed arm of fermentation tubes containing beef extract broth and various dissolved carbohydrates. Some growth is produced along the needle puncture in agar and gelatin stab cultures. In agar shake cultures no colonies develop in the depths of the media.

Numerous small colonies develop at the surface but none can be seen with a hand lens at a depth of 2 mm. or more.

Effect of light. In thickly sown plates of beef extract agar the bacteria were all killed by a 75 minute exposure to bright sun-light. In neutral pepper sap agar plates only about two-thirds of the organisms were killed, the darker color of the agar protecting them to some extent.

Acid production. In beef extract broth or agar containing 1 per cent dextrose, saccharose, maltose, or glycerine no evidence of acid production could be obtained. After the second day the medium in every case became gradually more alkaline. Following the observation that some alkaline substance was formed in the cultures, efforts were made to avoid the obscuring effect of the alkali on the production of acids. The first method tried was to increase the sugar content and use agar instead of broth. Three per cent dextroxe, saccharose, and lactose were added to beef extract agars. Brom thymol blue was then added as an indicator and sodium hydroxide solution was added until the media were neutral (grass green). The agar was then tubed and autoclaved 20 minutes under 10 pounds pressure. After a preliminary incubation, the tubes of agar were inoculated and the cultures incubated at 25° C. After 48 to 72 hours the agar about the bacterial growth had changed to the full alkaline color of the indicator. By the sixth day the color had returned to nearly neutral in the tubes containing dextrose and saccharose. By the eighth day the agar immediately around the bacterial growth had become decidedly acid yellow. This color gradually passed down through the agar until, by the end of a month, it was yellow throughout. The agar with lactose became deeper blue and the color never changed to yellow.

Acid production was not indicated when the organism was grown on beef extract broth plus 3 per cent of these sugars with the indicator added. The difference was probably due to the slower diffusion of the proteids through the agar.

By reducing the protein content of the broth, and consequently the production of alkali, it was found that very appreciable quantities of acid could be demonstrated with certain sugars. A one tenth per cent peptone solution plus 3 per cent dextrose or other sugar and brom thymol blue as indicator served beautifully for this purpose. In all cases there was a slight increase in alkalinity during the second and third days. By the fifth day the color had changed to yellow in the tubes containing dextrose or saccharose, and by the end of 3 weeks the reaction was orange to methyl red. With lactose the growth was not so rapid. Apparently the lactose was not used so long as any other nutrient was present, since no indication of acid production could be seen until the end of 2

weeks. After that the acidity increased rapidly, and the final reaction was similar to that obtained with dextrose and saccharose. With maltose the growth was fairly good but no acid was produced at the end of 6 weeks. With mannite or glycerine growth was very scanty. Apparently the organism was not able to use these substances.

Ammonia production. Ammonia was produced consistently in all media tested. In beef extract broth, in Dunham's solution, and in solutions of egg albumen the presence of ammonia was shown repeatedly by browning of filter paper wet with Nesler's solution and placed in the mouth of the culture flask or test tube. Futhermore, quantitative tests indicate that all the alkali formed is volatile and appears to be ammonia (gives brown color with Nesler's solution).

TABLE 1

True acidity and change in reaction of beef extract broth after six weeks incubation at 25° C.

		NaCl series				Beef extract broth series					
Acidity (Fuller's)		neutral	+5	+10	+15	+11	+12	+13	+14	+15	+16
Check	pH	8.5	7.4	5.6	4.8	6.4	6.3	5.8	5.4	5.1	5.0
	cc. N/20 NaOH to neutralize 10 cc.					1.35	1.7	2.0	2.2	2.4	2.6
Cultures, average of 4 flasks	pH	8.7	8.8	8.4	8.4	8.4	8.3	8.3	8.4	8.4	8.3
	cc. N/20 NaOH to neutralize 10 cc.					0.45	0.50	0.55	0.65	0.70	.087

Reaction of medium. The organism grows well in broth neutral to phenolphthalein and in broth with a moderately acid or moderately alkaline reaction. In one series, in which 1 per cent NaCl was added to the beef extract broth, growth was obtained in neutral, +5, and + 10, — 5, — 10, — 15, and —20 (Fuller's scale) but not in + 15 or — 25. Growth started first and appeared to be best in neutral and + 5. No growth was apparent in the — 15 until about the tenth day or in the — 20 until near the end of 3 weeks. In another series without the addition of NaCl growth was obtained in +10, + 11, + 12, + 13, + 14, + 15, and + 16, — 11, —12, —13, — 14, —15, —16, —17, and — 18. In the — 18 evidence of growth was not visible, but at the end of 30 days loop transfers to agar plates showed a large number of living

bacteria while in + 17 and — 19 or above the organisms were dead. It will be noted that growth occurred in the + 16 in this case whereas it did not occur in + 15 in the NaCl broth series. This is explained by the fact that the true acidity was greater in the latter case as shown in table 1.

Indol production. Eight tubes of Dunham's solution were inoculated and with two sterile tubes to serve as checks, were incubated at 25° C. At the end of 6 days all were tested for indol by adding at once 1 cc. of a 0.02 per cent $NaNO_3$ solution and 1 cc. of concentrated sulphuric acid. In all of the inoculated tubes a red color began to appear within a few minutes and gradually darkened to a brick red, while no red color appeared in either of the check tubes. At various times, also, positive results have been obtained with the vanillin test in cultures in Dunham's solution and in other media.

Phenol production. No indication of phenol production was obtained in any medium.

Reduction of nitrates. Beef extract agar plus 1 per cent KNO_3 did not show the presence of nitrite when tested on the second, fifth, and tenth days. Beef extract broth plus 1 per cent KNO_3 and 2 per cent saccharose gave positive tests on the sixth day. No growth could be obtained on nitrate media lacking organic nitrogen.

Color reduction. In litmus milk the color was at first destroyed but began to reappear at the surface of the liquid after 2 weeks. In litmus broth a similar behavior occurred. In broth tinted with methylene blue the color change was very striking. The color disappeared entirely from the depths of the liquid by the end of the fifth day, while it still remained intense in the upper centimeter. By the end of 10 days it had disappeared entirely, but it soon reappeared beginning at the surface of the liquid. In the beef extract agar plus 3 per cent saccharose and tinted with methylene blue the color disappeared throughout the depths of the agar by the sixth day. A thin film remained over the surface of the agar, even covering the bacterial mass.

Digestion of egg albumen. Ten grams of egg albumen were dissolved in 500 cc. of tap water. Fifty cc. of the solution were then placed in each of 10 flasks and autoclaved under 10 pounds pressure for 30 minutes. The albumen was coagulated into rather large lumps. Eight of the flasks were inoculated and, with the 2 checks, were incubated at 25° C. By the sixth day some evidence of digestion was visible; and by the end of 6 weeks practically all of the albumen had been dissolved, leaving a fairly clear yellowish liquid.

INOCULATIONS

Hundreds of plants have been successfully inoculated; but details will be given in only a few cases, which will serve to give a general idea of the methods used.

On July 16 an 8-day old bouillon culture was diluted with an equal volume of sterile water and sprayed with an atomizer onto seven young Pimento pepper plants. At the same time 7 similar plants were sprayed with sterile water as checks. All were covered with bell glasses for 72 hours. On the tenth day spots began to appear on all inoculated plants, at first as pale green or whitish pimples which collapsed and turned brown after a few days. By the fifteenth day infection was abundant. Forty-seven spots were counted on one leaf. All of the check plants remained healthy.

The organism was reisolated from one of the inoculated plants. Diseased tissue was cut out, dipped into strong alcohol, soaked 2 minutes in a 1–1000 solution of mercuric chloride, washed in sterile water, and then crushed in a tube of melted agar. Dilution cultures from this tube gave an abundance of the yellow colonies of the original organism with practically no contamination.

On September 22, 2 Tabasco pepper plants, one bell (Royal King), and 3 Chili pepper plants were sprayed with a water suspension of bacteria from a 4-day old agar culture isolated from spots on a bell pepper fruit. These plants with a like number of checks were then covered with bell glasses for 48 hours. On the eleventh day small whitish pimples began to appear on the under surface of the leaves on all inoculated plants. On the Tabasco leaves the spots were small (about 1 mm. in diameter). The spots did not enlarge to any appreciable extent later and the leaves were not distorted.

On March 14, 3 tomato (Globe) plants and two bell (Royal King) pepper plants were sprayed with a water suspension of bacteria from a 2-day old agar culture of the organism reisolated from a pepper leaf inoculated 2 months previously with bacteria from a pepper fruit spot. These 5 plants and a similar number of checks were covered 48 hours with bell glasses. On the tenth day infection was evident on the leaves and stems of the pepper plants and on the leaves of the tomato plants. A month later 155 spots were counted on a single leaflet from tomato and 299 on a somewhat larger pepper leaf. The spots on the tomato leaves were small (0.5 to 2 mm. in diameter), light straw yellow to dark brown in color, and raised on the lower surface or with a sunken center and slightly raised border. On pepper leaves the spots were very similar, but averaged slightly larger than on the tomato. Raised warty streaks, 1 mm. wide by 3 to 5 mm. long were produced on the pepper stems.

The organism was reisolated from spots on both pepper and tomato. The checks remained healthy.

All attempts to infect tomato fruits have given negative results.

A few infections have been obtained on both Pimento and Chili pepper fruits; the percentage of infections however, was very small. In the field natural infection was very abundant on fruits set during damp weather in the early summer of 1921. During the dryer weather of late summer it was difficult to find an infected fruit, although, at the time, infection was very abundant on the leaves. Observations indicate that there is only a short period in the development of the fruit when it is susceptible to infection and that is probably during the development of the lenticels on the young fruits soon after the petals fall. One difficulty in the way of studying artificial inoculation in young fruits is the fact that they drop off, if kept under very moist conditions.

When the bacteria are pricked into the flesh of green pepper fruits, they usually grow and kill the surrounding tissues; but the typical warty fruit spot is never produced.

Incubation period. The incubation period at ordinary summer temperatures is about ten to fifteen days. Signs of infection are usually visible on the younger leaves on the tenth day and they continue to appear until about the fifteenth day. A temperature of about 15° C. increased the incubation perod one third. Above 20° C. temperature does not appear to be the limiting factor. The acidity of the pepper sap doubtless accounts for the slow development of the spots. The acidity of the sap of the pepper plant, at least of the fruit, is very near the limit for growth of the bacteria.

PATHOLOGICAL HISTOLOGY

Materials used. For the early stages of infection bacterial suspensions were placed in droplets on the under surface of pepper leaves. The inoculated plants were then covered with bell glasses for 72 hours. On the third, seventh, eleventh, and twelfth days after inoculation the inoculated spots were cut out and killed in Durand's modification of Gilson's fluid or in a mixture of equal parts 95 per cent alcohol and glacial acetic acid. The killed material was then dehydrated, imbedded in paraffin, sectioned, and stained with various stains. The best staining was obtained with Haidenhain's iron-alum haematoxylin.

For the later stages spots in various stages of development on both leaves and fruits were killed, imbedded, sectioned and stained as for the early stages.

Bacteria within the host tissue. In material for the study of the very early stages of infection the bacteria were apparently lost in the killing

and dehydrating processes, so that the exact mode of penetration was not observed. In material killed on the eleventh and twelfth days many early stages of spot formation were found.

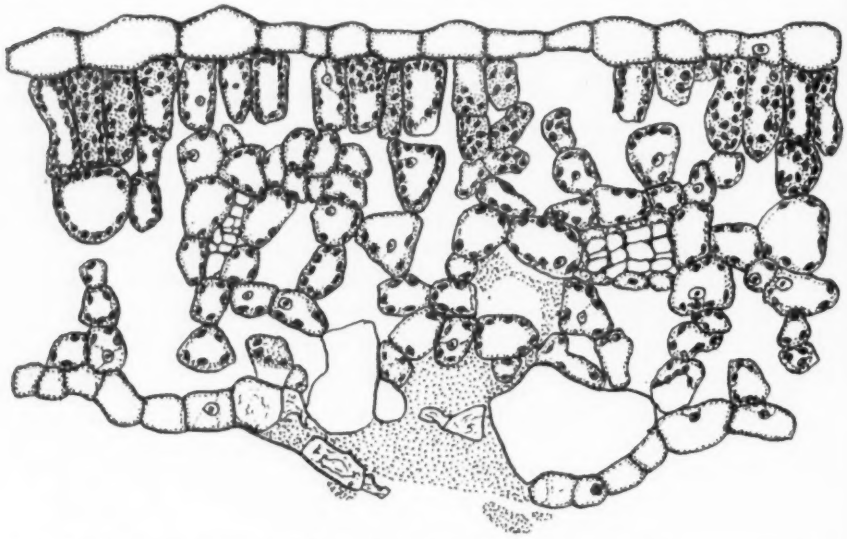


Fig. 3 Section of pepper leaf spot, showing an early stage of spot formation. The sub-stomatal cavity is filled with slimy bacterial growth (heavily stippled). The swelling of two mesophyll cells has broken the epidermis.

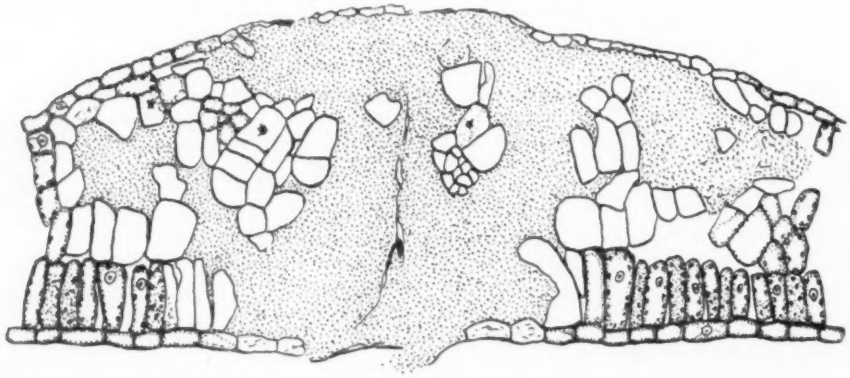


Fig. 4. Section of a leaf spot at a later stage than that shown in figure 3.

At this time slimy masses of bacteria were found within the stomatal cavity and in the adjoining intercellular spaces (Fig. 3). The cells in contact with this mass swell and break the epidermis, producing the pimples mentioned under "Inoculations." From this point the bacteria

spread to the intercellular spaces throughout the thickness of the leaf (Fig. 4), forcing the cells apart and finally killing and crushing them. After this the spot dries and the tissues collapse, the bacteria continuing to spread around the edges of the spot for some time thereafter (Fig. 5A).

The bacteria are imbedded in a mass of slimy material and especially in old spots, the individual bacteria can rarely be distinguished. This material seems to be hygroscopic and is doubtless responsible for a part of the swelling at certain stages; but a great part of the swelling is due to the actual enlargement of the host cells (Figs. 2, 5 A, 5 B). In young tissues in both leaves and fruits there is some stimulation to cell division. The hypertrophy is probably due to stimulation by small amounts of ammonia given off by the bacteria as suggested by Smith (7) in his discussion of crown gall. The final death of the cells may be due to excessive stimulation by the ammonia or to the coagulation and final digestion of the cell proteids by the bacteria. As the cells begin to swell the chloroplasts disappear. Later the cell membrane and the nucleus disappear. The nucleolus often persists after the cell is dead.

Early stages in the development of the fruit spots were not studied. In the later stages developments are very similar to those found in the leaf spots. The surface tissues collapse and become cracked and roughened (Fig. 5 B), The bacteria continue to advance laterally and toward the interior between the host cells. In old spots they often penetrate entirely through the flesh and into the seed cavity.

IDENTITY OF THE CAUSAL ORGANISM

The first mention of a bacterial spot of peppers, which I have been able to find, is in a short note on a leaf spot by Heald and Wolf (5) in their Plant Disease Survey of Texas. In 1918 Sherbakoff (6) gave quite a complete description of the disease as it occurs on the leaves and fruits of pepper in Florida. Recently a very similar disease of tomatoes was studied by Miss Doidge in South Africa. This disease was found to be caused by a bacterium for which Miss Doidge (2) suggested the name *Bacterium vesicatorium* n. sp. Shortly afterward a more complete description (3) of the disease and of the causal organism was published. Later Gardner and Hendrick (4) described a similar disease of tomatoes in Indiana. Although they refer to the work of Miss Doidge, and suggest that she has studied the same disease, they suggest another name, *Bacterium exitiosum* n. sp., for the causal organism.

The descriptions of the organism as given by Miss Doidge and by Gardner and Hendrick (4) are very similar, although the physiological reactions differ in several important points. The same has been found true in comparing the pepper organism with their descriptions. Doubt-

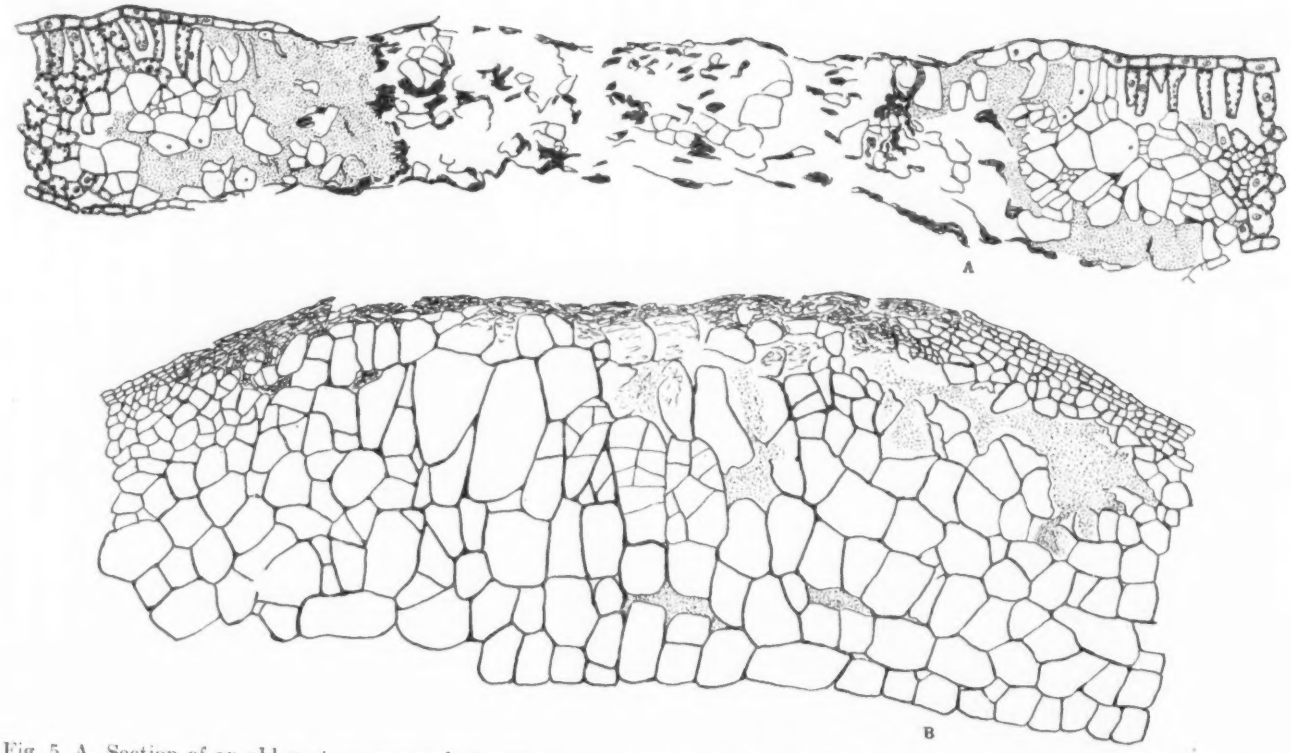


Fig. 5, A. Section of an old spot on pepper leaf. The bacteria are still growing in the border of the spot. Section through a fruit spot, showing the collapsed surface tissues and the bacterial masses which are forcing the cells apart in places.

less many, if not all, of the differences are due to variations in technique. Until a comparative study of the three organisms can be made, it seems useless, therefore, to suggest the identity of the pepper organism. According to my own results the group number should be *B.* 211. 2222523.

CONTROL MEASURES

The appearance of the disease in seed-beds where peppers had never been grown before was almost conclusive evidence that the bacteria had been brought in on the seed, and led to a closer study of this question.

Commercial pepper seed (Royal King variety) were germinated in sterile soil in the green house, and the seedlings were watched for appearance of the disease. Two lots of 300 seed each were soaked in water 6 hours. One lot was then treated 2 minutes with a 1–1000 solution of mercuric chloride, washed with sterile water, and planted in a large pot of sterile soil. The other lot was planted, without treatment in a similar pot of sterile soil. Of the treated seed 155 germinated from which all but 12 seedlings were healthy. Of the untreated lot 176 seeds germinated from which 126 seedlings developed spots on the cotyledons or on the hypocotyls. The greater part of these spots were produced by Colletotrichum, Gloeosporium, Macrosporium, and other fungi. The pepper spot bacteria were isolated from a number of the spots on the cotyledons and from one spot on a hypocotyl. Two such isolations were used in a number of successful inoculation experiments and in some of the physiological studies. Of the 12 spots on the seedlings from the treated seed 8 were produced by Macrosporium and the other four by Colletotrichum.

The mercuric chloride treatment was quite effective in control of the leaf spot, but in other tests the germination of the seed was severely injured. Therefore, more extensive studies of seed treatment must be made before definite recommendations can be published. Indications are, that if the disease can be kept out of the seed-bed, it is not likely to be troublesome in the field.

Spraying four times with Bordeaux mixture after the disease had become established in the field, reduced the disease considerably, but did not give complete control.

SUMMARY

1. The bacterial spot of pepper has been present in destructive form during the last three seasons in the Pimento growing region of Georgia.

2. The disease and the causal organism are described.

3. The disease is caused by a species of Bacterium very similar to *B. vesicatorium* Doidge and to *B. exitiosum* Gardner and Kendrick, but

it differs from the descriptions of these forms in some important physiological reactions.

4. The identity of the organism is still uncertain.

5. The causal bacteria are carried on the pepper seed.

6. Seed treatment with a 1–1000 solution of mercuric chloride is effective in controlling the disease, but it often injuries the germination of the pepper seed.

7. Spraying four times with Bordeaux mixture gave only partial control.

LITERATURE CITED

(1) CONN, H. J., H. A. HARDING, I. J. KLINGER, W. D. FROST, M. J. PRUCHA, and H. N. ATKINS. Report of the Committee on the Descriptive Chart for 1919. Jour. Bact. **5**: 127–143. 1920.

(2) DOIDGE, ETHEL M. A tomato canker. Jour. Dept. Agric. Union So. Africa **1**: 718–721. Fig. 1. 1920.

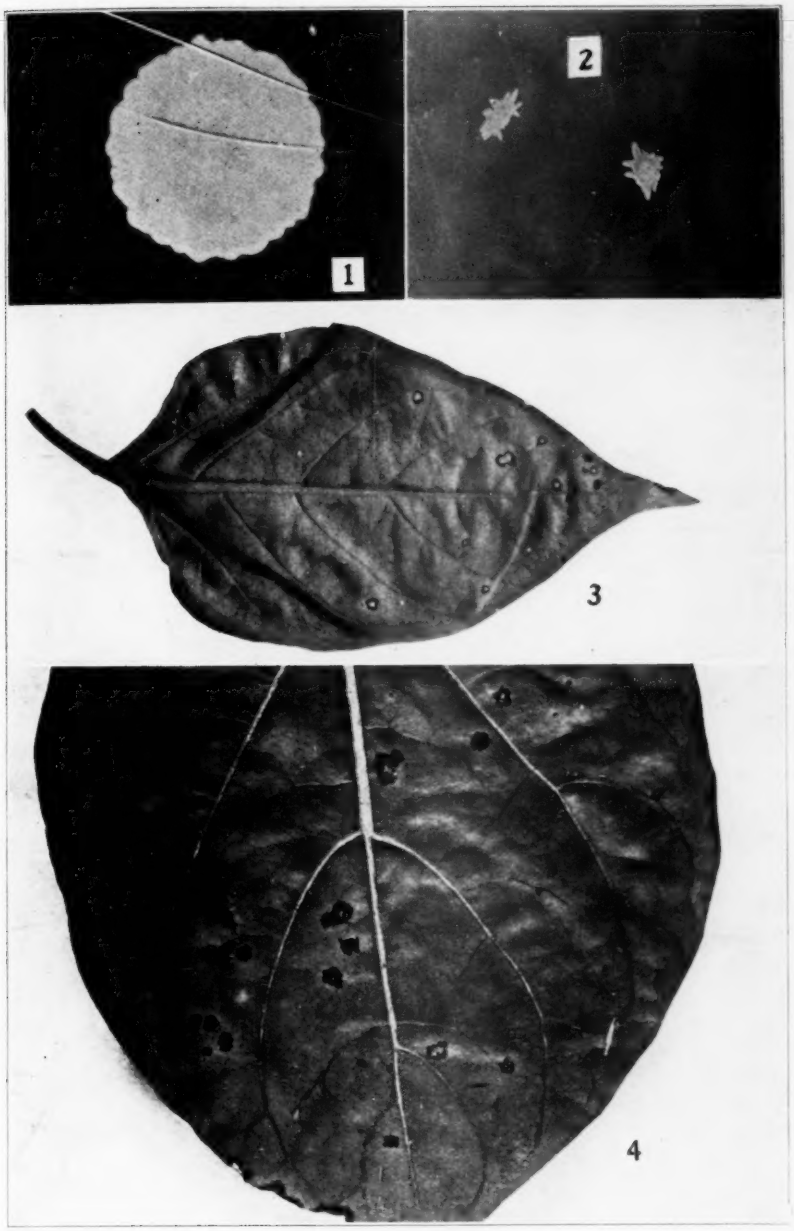
(3) ———— A tomato canker. Ann. Appl. Biol. **7**: 407–430. *Pl. 26, fig. 1–5.* 1921.

(4) GARDNER, M. W. and J. B. KENDRICK. Bacterial spot of tomato. Jour. Agric. Res. **21**: 123–156. *Pl. 24–28.* 1921.

(5) HEALD, F. D and F. A. WOLF. A plant disease survey in the vicinity of San Antonio, Texas. U. S. Dept. Agric. Bur. Plant Ind. Bull. 226: 1–129. *Pl. 1–19.* 1912.

(6) SHERBAKOFF, C. D. Report of the Associate Plant Pathologist. Florida Agric. Exp. Sta. Rept. 1916/1917: 66R–86R. *Fig. 3–8.* 1918.

(7) SMITH E F. Mechanism of tumor growth in crown gall. Jour. Agric. Res. **8**: 165–188. *Pl. 4–65.* 1917.



BACTERIAL SPOT OF PEPPER

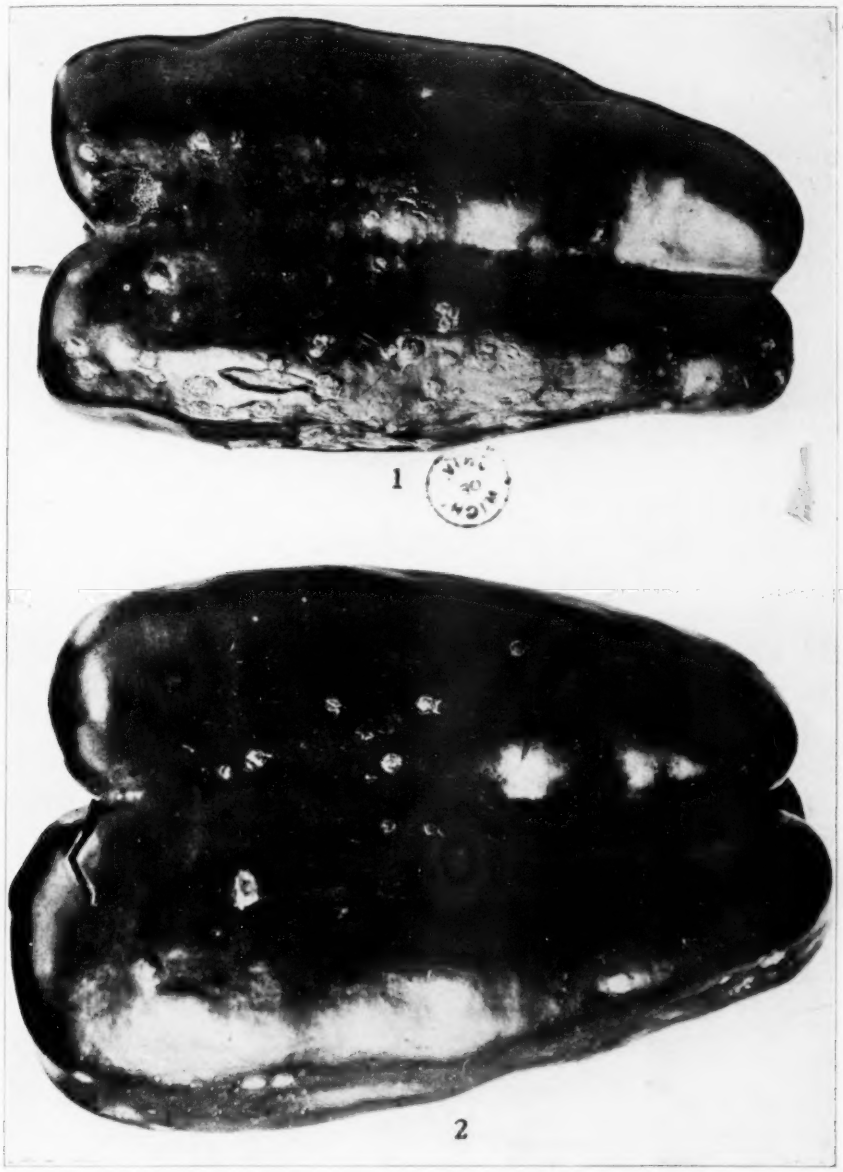
Fig. 1. Surface colony of pepper leaf spot bacteria in 27-day old agar plate culture × 3.

Fig. 2. Two submerged colonies from same culture. × 3.

Fig. 3. Bacterial spots on pepper leaf in early summer, showing light brown spots with darker borders.

Fig. 4. Bacterial spots on pepper leaf collected in the field November 11. Note that spots are dark brown. Natural size.

UNIV. OF MICH.



BACTERIAL SPOTS ON TWO FRUITS OF BELL (ROYAL KING) PEPPER. Natural size.

UNIV. OF MICH.

A NEW HOST FOR THE FIRE BLIGHT ORGANISM, BACILLUS AMYLOVORUS

LAETITIA M. SNOW

The first printed notice of fire blight was a report taken from a letter by William Denning, dated December 22, 1793, and published in the Transactions of the New York Society for the Promotion of Agricultural Arts and Manufactures (16). In this letter Mr. Denning stated that he had noticed for some years a "disorder" in apple orchards in the vicinity of the Hudson River above the Highlands, and that he had found it also attacking pear and quince trees.[1] Henry Ward Beecher in 1844 (7) quoted J. H. James and C. W. Elliott as reporting "blight" in various trees and shrubs, such as apple, pear, peach, quince, English hawthorn, privet, black birch, Spanish chestnut, elder and Calycanthus, although he himself had observed it only on fruit trees. In 1846 Downing (17) reported that he had seen it on several other trees besides the pear, for example the Ailanthus, Catalpa, and Spanish chestnut. Barry, in 1847 (6) added crab, walnut, and hickory to the list, and in the same year Eaton (18) suspected the Tartarian cherry of having the disease. Two years later (1849) James (23) noticed the same effect on common cherries and white currants besides several of the trees already mentioned. All the observations above noted were without convincing evidence as to the nature of the malady. In 1870 however, Hull (21) compared the disease in the pear and apple and proved that the "fungus is identical, by inoculation," which was the first experimental evidence offered.

In 1880 T. J. Burrill proved that the disease was due to bacteria, and reported the fact before the Illinois State Horticultural Society. The report in the Transactions of the Society (8) is, according to his own account the first published reference to the causal organism of pear blight and, it may also be added, to the bacterial infection of plants. His experiments included pear, apple, and quince. In the same year (9) he stated that "probably many other plants, among which we now name the butternut, Lombardy poplar, and American aspen, suffer from the same disease." He thought at first (11) that peach yellows was due to the fire blight bacterium, but later concluded (13) that the organisms were not "specifically identical." The butternut disease was charact-

[1] This reference is almost always given in pear blight literature and, with one exception, I have found it incorrectly cited from a Massachusetts publication.

erized, without proof, (10) as similar in every way to sun scald of apple, which he in turn identified with pear blight (12, 14). In one report (11) he stated that the Lombardy poplar was also destroyed by "these ferment producing agents," and that the aspen (*Populus tremuloides*) was similarly affected with blackening of limbs similar to pear and apple blight. Again no proof is offered of the identity of infecting organisms. In the following year, 1882, (13) he considered the Lombardy poplar disease "in cause and consequence" as "assuredly the same thing" as pear blight, stating that it had been communicated from one to the other by inoculations, but giving no details. "The aspen, the maples, the elms, the willows, the mountain ash, the lilac (leaves), the butternut, some herbaceous plants (peony, lettuce, potato?), etc., more or less suffer in the same way on account of the same, or an indistinguishable, voracious, little, but sufficiently potent, parasite." However, he gave no description of the disease, the culture reactions of the organisms, or of cross inoculations. From the above one would conclude that, while Burrill recognized the fact that certain plants were affected in a similar way by bacteria, he offered no, or insufficient evidence as to the identity of the attacking organisms.

Arthur in 1885 (2) reported the observation of the disease on "hawthorn," and successful inoculation experiments (4, p. 357) on service berry, English hawthorn (*Crataegus oxyacantha*), and an evergreen thorn (*Crataegus pyracantha*). A third article in the same year (3) added an observation of the disease on crab, confirmed Burrill's statement regarding its presence on mountain ash, and reported unsuccessful attempts to inoculate grape, raspberry, mulberry, peach, etc. A fourth article in 1885, (3, p. 375) stated that the effect of the fire blight organism upon peach was not the characteristic blight, but gummosis. Inoculations into black raspberry and green grapes yielded no results. Later (1907) Ralph E. Smith (30) attacked "peach blight" from another angle and concluded that it was not caused by the fire blight organism but by a fungus. In 1887 Arthur reported successful inoculation experiments on wild crab (*Pyrus coronaria*) but was unable to produce the disease on elderberry, abele poplar, or balm of Gilead.

In 1894 Sturgis (32) reported twig blight on Spaulding variety of plum, but was unsuccessful in obtaining either pure cultures of the organism or infection in apple, pear, or quince trees. He added, as the opinion of Arthur, that it was probably not identical with pear blight. In 1902 Jones (25) proved, by culture reactions and pathogenicity, that the pear blight organism was the cause of the blight of the Cheney plum (*Prunus americana nigra*), and quoted F. A. Waugh as reporting its presence in the Hortulana group also. In the following year Paddock

(28) proved by cross inoculations and culture reactions, that the apricot blight was caused by the same organism. He also thought that a blight on *Prunus simonii* was due to the same form, but offered no proof. Although Craig, (15) had reported in 1897 the presence of the disease on English hawthorn in Ottawa, in 1907 Edwards (19) stated that in Ontario neither English hawthorn nor any of the wild species of Crataegus had the disease. He found, however, that a double scarlet variety (*Crataegus oxyacantha* var. *splendens*) was subject to it, as was also a cut-leaved mountain ash (*Pyrus aucuparia* var. *laciniata*). In the same year (1907) Waite (33) added two plants to the list of "susceptibles," *Eriobotrya japonica* and *Heteromeles arbutifolia*. Whetzel and Stewart in 1909 (34) after listing the common hosts for the disease added "a few ornamentals," and stated that the form "appeared" to be on prunes. This was confirmed later (1915) by Jackson (22) by means of inoculations. O'Gara (27) in 1910 stated that loquat started one case of fire blight, but gave no evidence. In 1913 Hewitt (20), in his account of the history of the disease, stated that it was reported by various authors on pear, apple, quince, crab apple, peach, almond, plum, haw (several species of Crataegus), mountain ash, service berry, red raspberry, and black berry.[1] Reed in 1914 (29) specified among the "haws" *Crataegus crus-galli* as being specially susceptible to blossom blight. Munn in 1918 (26) was able to produce fire blight in strawberry blossoms by means of spray inoculation, but stated that it had not been found to occur in nature.

SUMMARY AS TO REPORTED HOSTS.

As the matter now stands, it has been proved, either by inoculations alone or in connection with culture reactions for the purpose of identification, that the fire blight organism is able to infect the following hosts: pear, apple, quince, service berry (*Amelanchier canadensis*), English hawthorn (*Crataegus oxyacantha*), evergreen thorn (*Crataegus pyracantha*), wild crab (*Pyrus coronaria*), cultivated crab, Cheney plum (*Prunus americana nigra*), apricot, prune, and strawberry. The claim for the Lombardy poplar is questioned. Other reports are presumably from observational evidence only, as they are given without any record of inoculation or the isolation and identification of the causal organism.

In the following report a new host, *Prunus triloba* var. *plèna*, is added to the list.

[1] In regard to several of these hosts the writer has not been able to find any other reference.

ISOLATION

While working in the Department of Plant Pathology of the University of Wisconsin in the summer of 1919, my attention was directed by Professor L. R. Jones, to a twig blight of the ornamental shrub *Prunus triloba* var. *plèna* in several localities near the University. A number of cultures were isolated from a clump of shrubs of this variety growing on Observatory Hill, and were labelled "B", but at this time no successful isolations were obtained from other specimens. Later (July 1920) a strain was isolated from a twig taken from a specimen growing in the garden of Professor Jones, and sent to Wellesley by Mrs. R. C. Williamson. This strain was labelled "J." Several strains were isolated at the same time as "B" from crab apple, Northwestern Greening apple, and from a species of hawthorn sent to the laboratory. The strain from the crab ("A") proved to be especially virulent, and was used, with a laboratory stock culture ("Wis. 165") for a control in the subsequent work. In the following descriptions the four strains (A, B, J, 165) will be understood to show like characters unless otherwise stated.

MORPHOLOGY AND CULTURE REACTIONS

Morphology.—The organism isolated from flowering plum is a short, motile, non-spore bearing rod, varying in size (J= 0.5 to 0.7 μ by 1 to 1.9 μ, B= 0.5 to 0.7 μ to 1.9 μ) and apparently becoming shorter with age. Shunk's method of staining revealed 1 to 2 flagella, chiefly at the ends, although occasionally one appeared to be attached at the side. The flagella were most abundant in A, rare in B, and fairly abundant in J and 165. All the strains were Gram negative (Sterling's method). No capsules were observed.

Agar streak.—Greyish white, J inclined to be brownish when old. Opacity varying with amount of growth; growth more vigorous on potato agar than on nutrient agar and inclined to be somewhat darker in color. Streak slightly depressed in the center, edge clearly defined; smooth and shiny. Growth of B and J somewhat more abundant than A and 165.

Agar colonies.—Round, finely granular to the edge, which is entire at first but may be wavy later; shiny, smooth, usually slightly raised. If deep, may be round or lenticular. Colonies of 165 were not noted.

Gelatin stab.—White in A, 165 and B; slightly yellowish or brownish in J. Growth best at top. Liquefaction so slight at the end of 30 days as to be questionable; not discernible in J. This result was obtained in 1921, and possibly may be correlated with the loss of peptonizing power to be noted below. Arthur (1) reported no liquefaction in two weeks, but other writers record slight liquefaction.

Litmus milk.—In 1919, immediately after isolation, A and B showed a faintly acid reaction after 3 days, becoming neutral after a week, with a soft coagulum, and a little peptonization at the top. After 2 weeks the soft curd was alkaline and peptonization had proceeded one third to one half way down the tubes.

In 1921 the action was very slow; 165 showed no change after 12 days while the others had started to change. No acid was evident. All the cultures became slightly alkaline and somewhat faded at the bottom. B and J were a little more alkaline than A and 165. No coagulation was noted after 12 days, but by 30 days a soft coagulum was present, least firm in 165. No peptonization occurred. This change in reaction must have been due to some common cause, for it occurred in A as well as in B, and in as much as the 1921 reactions of A and B agreed with those of J and 165, which were not tested in 1919, long cultivation is suggested as the causal factor.

Sugar broths.—No gas was produced at any time. In each case the culture was cloudy in the outer part of Dunham's fermentation tube, and clear in the inner tube. There was little sediment and a slight pellicle (a mere ring in J). The total acidity was tested by titration with the following results.:

Dextrose	+7.6 to +13.5	Fuller's scale	(165, A, B, J)
Maltose	+2.0 to + 6.0	" "	(A, B, J)
Lactose	−7.0 to + 1.0	" "	(A, B, J)
Sucrose	+6.8 to + 7.5	" "	(A, B)

Nitrate reduction.—The experiment lasted nearly 4 weeks and the tubes were tested after 4, 9, and 27 days. No nitrogen was evolved and no nitrites were formed, but a moderate amount of ammonia was produced, the most by B. Jones (24) and Stewart (31) state that no ammonia is formed. These experiments were repeated several times with the same result and the writer feels certain that nitrates are reduced to ammonia by strains A, B, J, and 165, but without nitrites or nitrogen being produced.

Indol and hydrogen sulphide.—In Dunham's solution, after 2 weeks, no hydrogen sulphide or indol were produced. These results agree with those of Jones (25) as regards hydrogen sulphide, but differ with respect to indol. Jones (24) and Stewart (31), however, report no indol formation.

Potato.—For this work plugs were cut from raw potatoes, placed in tubes and sterilized. The first two sets were sterilized by the discontinuous method, and the third in the autoclave. The method of steriliza tion appeared to be unimportant. Three series were run, one in 1919

and two in 1922. The first two showed considerable variation both in the cultures of the same strain and in different strains. In the third set there was some slight variation between the different cultures of the strains, but the different strains showed almost absolutely identical variations. In the last series B was, on the whole, somewhat lighter in color and more abundant than the others. At first all were dirty white, becoming somewhat darker with age, J tending toward a brownish tint and the other three toward yellow. In all, the growth was scanty to fairly abundant, smooth and shiny at the start, becoming drier with age. In the first series A had a distinctly sour odor, which was not noted in B, (165 and J were not included in this series). In the second series the same odor was noticed in A and 165, while that of B and J was very faint and difficult to characterize. This peculiar, faint odor was characteristic of all the cultures in the third set. I cannot account for this variation unless it was due to the age of the cultures used in transferring. In the third set all cultures were selected as good, vigorous ones of the same age. Many authors have noted a characteristic odor (variously described) in blighted plants. Arthur (5) calls it a weak, peculiar odor, not like decay. Others speak of it as "sour." The growth on potato agrees more nearly with that described by Arthur (1) than with that described by other authorities.

PATHOGENICITY

Immediately after isolation B was pathogenic for pear (green fruits) but inoculation into fairly young shoots of the flowering plum yielded no results. The fact that this was done about the middle of the summer may account for the negative results, but it has not been possible thus far to make inoculations in the early spring. After a year's cultivation one culture of this strain was very slightly pathogenic for pear. This sub-strain was lost during experimentation, and the work was continued with a culture, which when tested in 1920, proved to be non-virulent. A had remained strongly virulent after 2 years of cultivation. J was only weakly pathogenic for pear when isolated and lost virulence almost entirely after a year's cultivation. Wis. 165 was not found to be virulent when obtained from the laboratory.

SUMMARY

1. Strains B and J, isolated from blighted twigs of *Prunus triloba* var. *plèna*, were found to be pathogenic for green pear fruits with characteristic blighting and formation of milky exudate.

2. Compared with strain A (isolated from crab apple) and 165 (from the laboratory of Plant Pathology, University of Wisconsin) the culture

reactions of B and J agree. They also agree, in the main, with reactions given by other investigators. The following exceptions may be noted:

(a) In the formation of ammonia from nitrate all four strains differ from previous reports.

(b) During cultivation the power to digest casein was lost, with a corresponding slight or no liquefaction of gelatin.

(c) No indol was formed by any of the strains.

(d) The color of the growth on potato differs from that reported by several investigators, but agrees with that given by Arthur.

3. During cultivation 165, B and J lost their virulence while A remained strongly pathogenic for pear.

I wish to extend my thanks to Professor L. R. Jones, Department of Plant Pathology, University of Wisconsin, for personal suggestions and helpful criticisms and for the departmental courtesy extended to me during the progress of the investigation. My thanks are also due Professor Franz Aust of the Department of Horticulture for the identification of the host plant.

WELLESLEY COLLEGE,
WELLESLEY, MASS.

LITERATURE CITED

(1) ARTHUR, J. C. History and biology of pear blight. Proc. Phila. Acad. Nat. Sci. **38**: 322–341. *Pl. 3.* 1886.

(2) ———— [Pear blight]. New York (Geneva) Agric. Exp. Sta. Bull. (n. ser.) 2: 1–4. 1885.

(3) ———— Pear blight and its cause. Amer. Nat. **19**: 1177–1185. 1885.

(4) ———— Report of the Botanist, New York (Geneva) Agric. Exp. Sta. Ann. Rept. (1884) **3**: 353–385. *Fig. 1–5.* 1885.

(5) ———— Report of the Botanist, New York (Geneva) Agric. Exp. Sta. Rept. (1885) **4**: 241–296. *Fig. 1–10.* 1886.

(6) BARRY, PATRICK. Insect blight. Genesee Farmer **8**: 218–219. 1847.

(7) BEECHER, H. W. The blight in the pear tree; its cause and a remedy for it. Mag. Hortic. **10**: 441–456. 1844.

(8) BURRILL, T. J. Pear and apple tree blight. Trans. Illinois State Hortic. Soc. **14**: 157–167. 1880.

(9) ————Anthrax of fruit trees or the so-called fire-blight of pear, and the twight blight of apple trees. Proc. Amer. Assoc. Adv. Sci. **29**: 583–597. 1880.

(10) ————Blight of pear and apple trees. Illinois Indus. Univ. 10th Rept. (2 yrs. ending Aug. 1880). P. 62–84. 1880.

(11) ———— Bacteria as a cause of disease in plants. Amer. Nat. **15**: 527–531. 1881.

(12) ———— "Sun scald." Trans. Illinois State Hortic. Soc. **15**: 157–159. 1881.

(13) ———— Have we any new light on pear blight or yellows? 11th Ann. Rept. Michigan State Hortic. Soc. P. 133–139. 1881–1882.

(14) ———— Trunks of apple trees. Trans. Wisconsin State Hortic Soc. **15**: 215–229. 1885.

(15) CRAIG, J. Diseases of fruits. Rept. Minister Agric. Canadian Exp. Farm **1896**: 168–173. 1897.

(16) DENNING, WILLIAM. On the decay of apple trees. (From a letter to Mr. Mitchell, dated Dec. 22, 1793) Trans. New York Soc. Prom. Agric. Arts and Manu. 2nd ed. rev. **1**: 185–187 (Part 2). 1801.

(17) DOWNING, A. J. Blight of pear tree. Hortic. and Jour. Rural Art and Rural Taste **1**: 58–62. 1846–1847.

(18) EATON, L. C. A review of opinions on pear tree blight. Hortic. and Jour. Rural Art and Rural Taste **1**: 459–463, 495–500. July, 1846 to June, 1847.

(19) EDWARDS, S. F. Bacterium amylovorum. 32nd Ann. Rept. Ontario Agric. Col. **1906**: 136–137. *Fig. 1–5.* 1907.

(20) HEWITT, J. L. Twig blight and blossom blight of the apple. Arkansas Agric. Exp. Sta. Bull. **113**: 493–505. 1913.

(21) HULL, E. S. Twig blight of the apple. Root pruning trees in the summer. Trans. Illinois State Hortic. Soc. **4**: 220–223. 1870.

(22) JACKSON, H. S. Notes and observations on plant diseases. Fire blight on prune. Bien. Crop Pest and Hortic. Rept. Oregon Agric. Exp. Sta. **2**: 279–281. 1915.

(23) JAMES, J. H. The blight in pear trees. Mag. Hortic. **15**: 13–23. 1849.

(24) JONES, D. H. Bacterial blight of apple, pear and quince trees. Ontario Agric. Col. Bull. **176**: 1–63. *1 pl. (Col.), fig. 1–58.* 1909.

(25) JONES, L. R. Studies upon plum blight. Centralb. Bakt. Parasitenk. u. Infektionskrankh. Abt. 11, **9**: 835–841. 1902.

(26) MUNN, M. T. Pathogenicity of Bacillus amylovorus (Burr.) Trev. for blossoms of the strawberry (Fragaria sp.). Phytopath. **8**: 33. 1918.

(27) O'GARA, P. J. Control of pear blight on the Pacific coast. Better Fruit **5**: 30–43, 49–57. 1910.

(28) PADDOCK, W. An apricot blight. Colorado Agric. Exp. Sta. Bull. **84**: 5–14. *Pl. 1–2, fig. 1–2.* 1903.

(29) REED, G. M. An unusual outbreak of apple blossom blight. Phytopath. **4**: 27–30. 1914.

(30) SMITH R. E. California peach blight. California Agric. Exp. Sta. Bull. 191. P. 74–100. *Fig. 1–16.* 1907.

(31) STEWART, V. B. The fire blight disease in nursery stock. New York (Cornell) Agric. Exp. Sta. Bull. 329: 314–372. *Fig. 112–126.* 1913. Bibliography, p. 368–371.

(32) STURGIS, W. C. A "fire blight" of plum trees. Connecticut Agric. Exp. Sta. Rept. **18**: 117–118. 1894.

(33) WAITE, M. B. A new native host for pear blight. Science **25**: 286–287. 1907.

(34) WHETZEL, H. H. and V. B. STEWART. Fire blight of pears, apples, quinces, etc. New York (Cornell) Agric. Exp. Sta. Bull. 272: 31–52. *Fig. 5–22.* 1909.

YEAST-SPOT OF LIMA BEANS[1]

S. A. Wingard

With Four Figures in the Text

Examination in the fall of 1921 of diseased Lima beans, *Phaseolus lunatus* L., from eastern and central Virginia revealed the presence of the vegetative cells, asci and ascospores of a yeast which was constantly associated with the affected tissue. The organism has been readily isolated from several lots of beans by the following method: The affected seed were immersed for about a half minute in 95 per cent alcohol, some of the granular tissue removed from the lesion on a sterile needle and placed in a tube of sterile water. After shaking vigorously to break up the clumps of ascospores, several drops of the suspension were transferred to petri dishes, which were then poured with beer wort agar. These platings invariably produced numerous yeast colonies free of all contamination.

The yeast under discussion clearly falls under the genus Nematospora which was described by Peglion[2] in 1901. There are two species described in this genus, both of which are plant pathogens namely *Nematospora coryli* Peg. described from hazel nuts in Italy by Peglion in 1901, and *Nematospora lycopersici* Sch. described from tomato fruit by Schneider[3] in 1916. The form on Lima beans differs from these in certain characters and is apparently an undescribed species. The name *Nematospora phaseoli* is therefore proposed. A description of it is appended.

DIAGNOSIS

Nematospora phaseoli n. sp.

Form. The cells show a wide variation in form. The elliptical and spherical types as shown in figure 1, A predominate in young cultures. Other forms are found in varying numbers depending upon the conditions under which the cultures are grown. Mycelium-like strands, and cells

[1] Paper 59 from the Department of Plant Pathology, Virginia Agricultural Experiment Station. Read at the Toronto Meeting of the American Phytopathological Society, December, 1921. See abstract in Phytopathology **12**: 47. 1922.

[2] Peglion, Vittorio. Über die Nematospora coryli Pegl. Centralbl. f. Bakt. Abt. 2, **7**: 754–761. *1 pl.* 1901.

[3] Schneider, Albert. A parasitic saccharomycete of tomato. Phytopathology **6**: 395–399, *4 fig.* 1916. Further note on a parasitic saccharomycete of tomato, Phytopathology **7**: 52–53. 1917.

in the shape of tennis rackets, walking sticks, etc., are not uncommon in 24 hour cultures.

Arrangement. The organisms commonly occur in groups. In young cultures 1 to 5 buds are usually attached to the mother cell as shown in figure 1, A. In some cases the mother cell sends out a group of bud cells from one end and a mycelium like strand from the other. These strands are septate and, in most cases, form buds at the crosswalls.

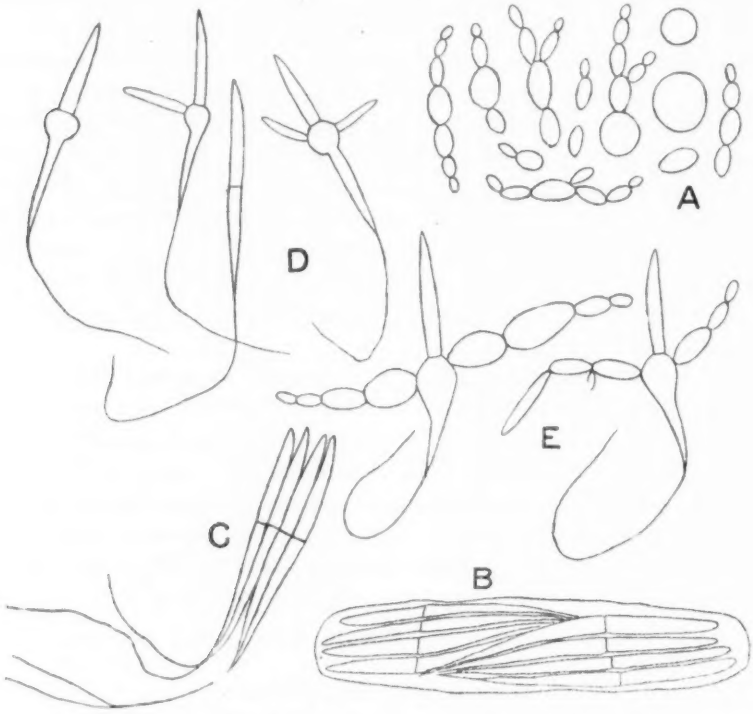


Fig. 1. Nematospora phaseoli. A, Vegetative cells, showing the characteristic budding; B, mature ascus, showing the arrangement of the ascospores; C, a cluster of four ascospores clinging together after being released from the ascus; D, germinating ascospores, showing the mode of germination in which a typical germ tube is produced; E, germinating ascospores, showing the budding mode of germination.

Size. Elliptical cells in young cultures vary in length from 8 to 14 μ and in diameter from 6 to 10 μ. The mature spherical cells are 20 μ in diameter, and the mycelium-like strands vary in length from 90 to 140 μ and in diameter from 2.5 to 3.5 μ.

Asci and ascospores. Asci and ascospores are produced in great numbers in the lesions on Lima bean seed and also on favorable nutrient media. The asci are cylindrical with rounded ends (Fig. 1, B and Fig.

2, C), 60–85 μ × 10–12 μ; ascospores 8, in two groups of 4, 40–46 μ × 2.5–3 μ, slender, 1-septate, slightly ridged at septum, apex acute, base extended into a slender, non-motile whip, which averages about one and one-fourth times the spore length (Fig. 1, D, and Fig. 2, D).

Ascospore germination. Two modes of spore germination have been observed. In the first and most common type, the basal cell swells at the transverse septum forming a sphere about 6 μ in diameter from which a germ tube protrudes and grows into a mycelium-like strand of considerable length, with crosswalls and branches, which give it the appearance of a true mycelium (Fig. 1, D). In the second the sphere is formed as in the preceding but instead of sending out a septate strand, spherical cells (Fig. 1, E) bud off from it.

Staining Reactions. The vegetative cells, asci and ascospores stain readily with the ordinary stains; the reaction to the Gram stain is positive.

CULTURAL CHARACTERISTICS

No extensive study has been made of the growth of the organism on different culture media, but it has been grown on several media to determine which are most favorable for its development. In general it grows best on media that are most favorable for the growth of the ordinary yeast, that is, on beer wort agar, and on fresh vegetable material such as garden beets, Irish potatoes and sweet potatoes.

Beer wort agar. On beer wort agar plates at a temperature of 30° C., the colonies appear on the second day. They are convex, circular, with entire margins and smooth surface, opaque, dull. The internal structure is finely granular (Fig. 2, B). The colony is cream colored at first but gradually turns brown with age. By the end of the seventh day the colony has attained its maximum size, which is from 5 to 10 mm. in diameter (Fig. 2, A). Asci and ascospores are produced in great numbers in 48 to 72 hours from the time of plating. After about three weeks, the colony becomes surrounded by a border of mycelial growth. A second crop of asci is produced on these mycelial branches.

On beer wort agar slants there is an abundant growth, which is slightly filiform in appearance, raised, dull, cream colored, opaque, contoured, with a butyrous consistency (Fig. 2, G). An alcoholic odor is apparent.

Whey agar. The organism made a good growth on one lot of this medium, but failed to grow on other lots which were prepared later.

Corn meal. The yeast develops poorly on corn meal agar, but grows well on steamed corn meal and water.

Potato and beet. Fresh garden beets, Irish potatoes, and sweet potatoes have proved to be very favorable media (Fig. 2, E and F). In some cases

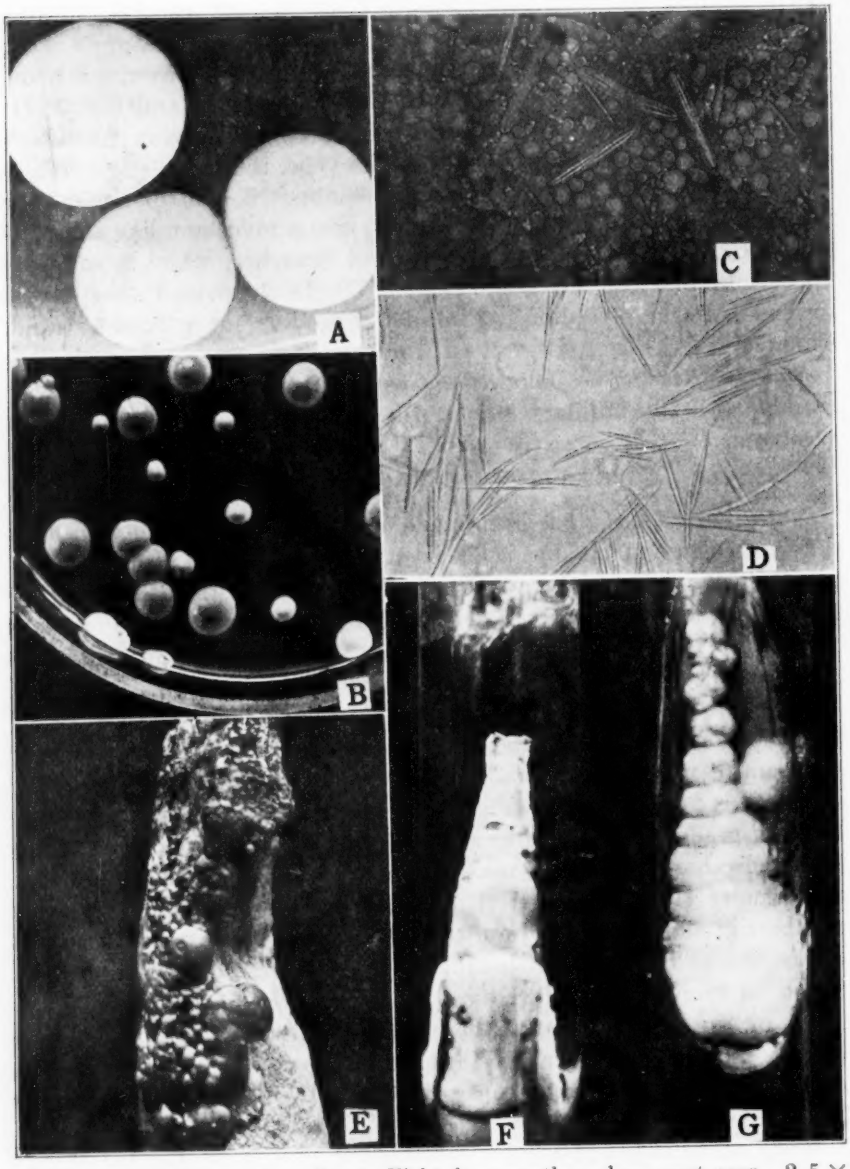


Fig. 2. Nematospora phaseoli. A, Eight-day growth on beer wort agar. 2.5 ×. B, Ten-day growth on beer wort agar at 30° C., about natural size. C, Vegetative cells, asci and ascospores, about 200 ×; D, Mature ascospores, about 400 ×. E, One-month growth on Irish potato, 2.5 ×. F, Eight-day growth on sweet potato, 1.2 ×. G, Eight-day growth on beer wort agar, 1.2 ×.

the slants are completely covered by the growth of the yeast in 48 hours from the time of inoculation.

Beef peptone agar. This is a very poor medium for the growth of the organism. Colonies are produced but they never attain a diameter of more than 1 to 2 mm. Asci and ascospores occur very sparingly in these colonies.

Czapek agar. No growth is made on this medium.

TEMPERATURE RELATIONS

The optimum temperature for growth has not been carefully determined but it appears to be about 30° C. The organism makes a very poor growth and forms asci and ascospores very slowly at a temperature of 18° C.

APPEARANCE OF THE DISEASE

The disease occurs on the seed in the pod. It causes numerous dark sunken areas on the cotyledons. The organism is apparently able to attack the seed at any time during development, but the most severe injury seems to result when infection takes place before the seed is half grown. In case of early infection the seed may either die prematurely or fail to grow to normal size (Fig. 3, A). Affected seed vary from one-tenth to natural size. In the majority of cases the testa remains unbroken, the infected spot being dark brown and somewhat sunken and wrinkled (Fig. 3, C). This, however, is not always true, since in some cases the testa is ruptured the disease manifesting itself in the form of crater-like lesions on the cotyledons (Fig. 3, B). The tissue in the lesions is grayish-brown in color, and granular in texture. In the lesions are found great masses of ascospores, and a small number of vegetative cells and young asci.

The disease has only been observed on the seed up to the present time. Pods that appear perfectly healthy may contain badly diseased seed.

DISTRIBUTION

The original specimen of this disease was collected in York County, Virginia, October 15, 1921, by Mr. A. G. Smith, Jr.[1] Additional collections were made by Mr. Smith in this and six other counties in the State. Specimens of the disease have been collected from the following counties: York, Henrico, King and Queen, King William, Essex, Albemarle and Dinwiddie. The ease with which the specimens were found indicates that the disease is rather prevalent and widely distributed.

[1] The writer is greatly indebted to Mr. A. G. Smith, Jr., Specialist in Vegetable Gardening, Virginia Extension Division, for collecting affected material in the field

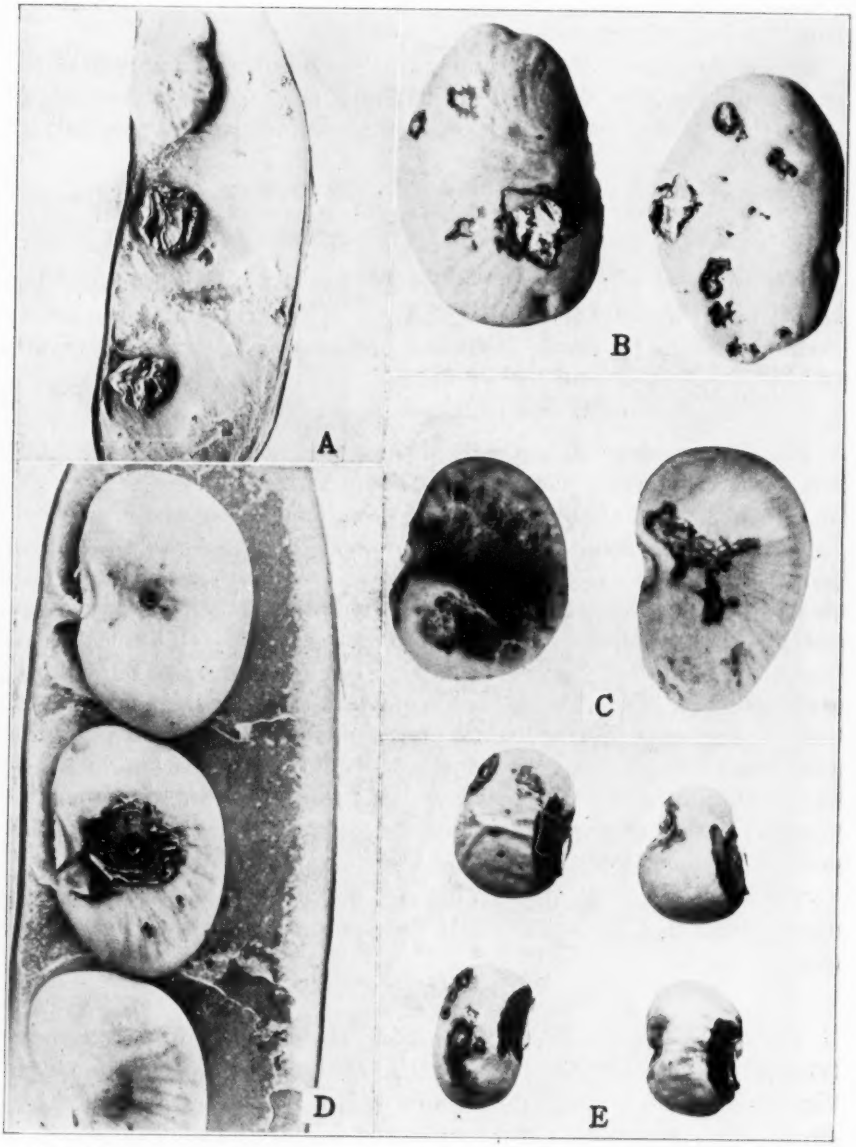


Fig. 3 Nematospora phaseoli. A, Lima bean pod and seed showing the result of natural infection in the field, 1.5 ×. B, Natural infection showing crater-like lesions and ruptured testa, 3 ×. C, Natural infection on Lima bean seed, resulting in dark brown sunken spots on the cotyledons in which the testa remains intact, 3 ×. D, Artificial infection on Lima bean seed, 2.5 ×. E, Yeast-spot infection on Blackeye cowpea seed, 3 ×.

The percentage of infection varied from a trace to as much as 60 per cent of the crop.

Affected seed of several different varieties of Lima beans have been collected, but the small Lima or sieva type seems to be especially susceptible to the disease. One specimen of blackeye cowpea has been collected which was affected with the same or a very similar yeast (Fig. 3, E).

PATHOGENICITY

The pathogenicity of the yeast on Lima beans has been demonstrated repeatedly in the greenhouse. Spraying the young pods by means of

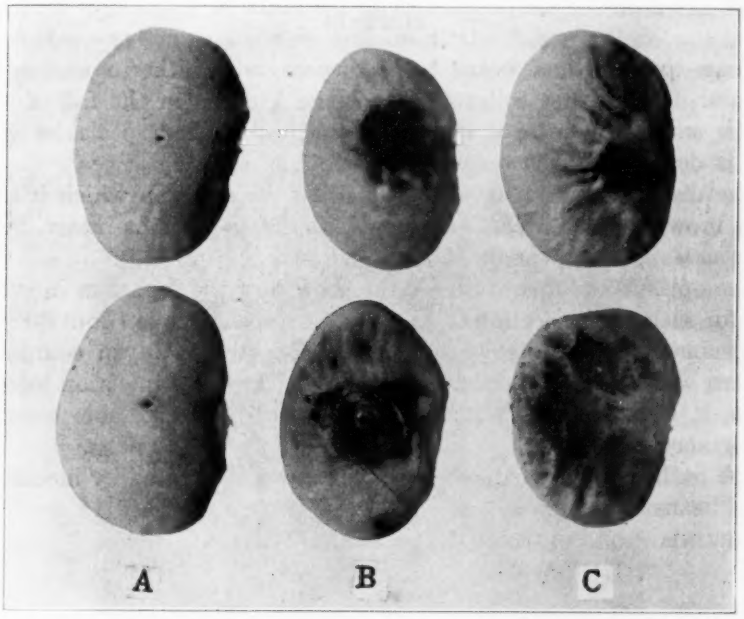


Fig. 4. Nematospora phaseoli. A, Two Lima bean seed which were used as controls in a greenhouse inoculation experiment, 2.5 ×. B, Two Lima bean seed which were aerificially inoculated in the greenhouse, 2.5 ×. C, Two Lima bean seed showing natural infection in the field, 2.5 ×.

an atomizer with a water suspension of a pure culture of the organism failed to produce infection. Negative results were also obtained when the pods were smeared with a pure culture of the yeast grown on agar. The seed was readily infected when the pods were punctured with a needle, dipped into a pure culture of the yeast. Additional inoculations of the seed made in a similar manner resulted in infections closely resembling those produced under natural conditions (Fig. 3, D and

Fig. 4, B). The characteristic symptoms are evident within two or three days after inoculation. They are quite conspicuous within 7 to 10 days.

No infection was produced on garden beans, *Phaseolus vulgaris*, in the greenhouse. Tomato fruits in different stages of development were inoculated in the greenhouse by puncturing, but none of them showed signs of decay unless allowed to become over ripe. In the few cases where decay was noted, an examination revealed the presence of other fungi. This yeast, therefore, seems to be only weakly parasitic on tomatoes. It is able however to survive in the green fruit where asci and ascospores are formed as soon as the tomato ripens.

SUMMARY

Yeast-spot of lima beans and cowpeas is a hitherto undescribed disease. It was first collected in eastern Virginia in the fall of 1921.

The causal organism is apparently an undescribed species of yeast, and is described as *Nematospora phaseoli*, n. sp.

The disease appears to be restricted to the seed, on which it forms dark brown sunken areas. As a rule, the testa remains intact, but in some cases it is ruptured.

The optimum temperature for the growth of the organism in culture and for successful infection of Lima beans appears to be about 30° C.

Specimens of the disease have been collected in seven counties in eastern and central Virginia. From the severity of infection in many cases, it is likely that the disease may be of considerable economic importance.

The pathogenicity of the yeast has been demonstrated by inoculating Lima beans in the greenhouse.

VIRGINIA AGRICULTURAL EXPERIMENT STATION.

PHYTOPATHOLOGICAL NOTES

Plant pathology in Crimea.—Apple scab (*Venturia inaequalis*) and pear scab (*V. pyri*) are serious diseases in Crimea as well as in the United States. The brown rot organism differentiated into *Sclerotinia cineria* and *S. fructigena* is exceedingly serious to both fruit and trees. The latter form only is supposed to be responsible for injury to trees. The writer was shown a cherry orchard at the Salgir station which was

Fig. 1. Injury to fruit trees in Crimea caused by the brown rot fungus.

being killed by this organism. The fungus advances progressively from fruit to twigs and then to the main limbs and trunk much as does our pear blight organism. The accompanying photograph (Fig. 1) made under the writer's direction illustrates the method of attack and damage wrought. The disease is most serious on sweet cherries, and to trees under poor culture conditions. Such conditions as now obtain are to a large extent due to civil wars.

In the limestone country north of the Crimean mountains chlorosis is serious. It is also to be noted that this region is entirely irrigated. At the Salgir station some interesting experiments in attempting to control chlorosis by injecting chemicals have been conducted. The results are said to have been published in Russian journals. A mixture of $FeSO_4$, K_2SO_4 and $MgSO_4$ dry and in solution were injected into trees through holes bored into the trunk. There were no untreated controls. The writer observed that above the reported point of injection the foliage was normal green, while below that point it was yellow.

The method of pruning trees has frequently been responsible for their death from heart rots and cankers. The trees in the older orchards have a large number of main branches. As a result the tops are crowded and the fruit is borne at the ends of spindling branches, which in turn reduces the yield and endangers them to breaking. The growers are now sawing off limbs five to seven inches in diameter, the wounds being left untreated. Such trees generally become infected and eventually die. *Nectria ditrissima* is very serious.

Plant pests on the south slope of the mountains are frequently different from those on the north. *Gymnosporangium sabinae* is quite serious on pears. It is noteworthy that three species of Juniper are quite abundant in the mountains. *Sphaerotheca pannosa* was observed on peaches.

Insect pests cause greater damage to fruit in Crimea than fungi. The codling moth is most serious. There are many leaf-eating caterpillars and other insects not recorded in American works on fruit pests. They are particularly serious now due to the lack of insecticides.

The methods of pest control are quite backward. The sprays used when obtainable are Bordeaux mixture and Paris green. Lime-sulphur is only known as a dormant insecticide and self-boiled lime-sulphur as a spray for mildew on gooseberry. Last spring the orchardists were unable to spray because of the failure to secure shipments from Moscow of copper sulfate for the preparation of Bordeaux mixture. There are deposits of sulphur in Crimea and in the Caucasus, and abundant lime throughout Crimea so that lime-sulphur might have been made, had the growers known of its use. Power sprayers are unknown in Crimea. Indeed I was informed that there was not a power sprayer in all Russia. Small hand sprayers are used. These ordinarily are operated by a force of six men—two to pull the sprayer, two to pump, and two to spray. The pear trees north of the mountains reach a great height. The tops are sprayed from tall ladders.

The plant pathologist at Nikitski Botanical Gardens, Dr. Deckenbach, who speaks English quite well, is acquainted with American

literature. He is starting experiments in the use of lime-sulphur. The communication between scientists in Moscow and Crimea has been very poor. Dr. Deckenbach travelled north with the writer and in Moscow read his obituary notice written the year before by Prof. Jaczewski who recently visited America.

There is considerable neglect of orchards due to unavoidable disorganization incident to war and revolution but the main problem in Russia is to improve the normal pre-war methods. These methods were behind those in this country due to the backwardness of the peasants, and to the corruption, incompetence, and indifference of the former ruling class.

With the vigorous cooperation of the Russian government the scientists are making a campaign to improve the agricultural methods of Russia. They deserve all the help which American scientists can give them. They especially need scientific literature.

I found no American scientific literature in Petrograd and Moscow, for the period from 1917 to 1921 and very few publications since 1913. A few publications had found their way into Crimea during its occupation by counter-revolutionary forces. One scientist showed me two agricultural bulletins which he was guarding as though they were valuable treasures. Fortunately we now have a scientific committee at work collecting literature for the Russian scientists. I hope that American scientists will be generous in their contributions to this committee—H. W. TRUESDELL.

Mistletoe and Smelter smoke.—In 1921, the writer examined an area of the woodland type which for four years had been exposed to smoke from the big copper smelter at Clarkdale, Arizona. This smoke was said to contain compounds of sulphur and arsenic. Scattered over this area were many one-seeded junipers (*Juniperus monosperma*); close to the smelter the trees were dead but further away many were injured in various degrees but not yet killed. Some fifty of these living junipers had once been infected by mistletoe (*Phorodendron juniperinum*) but at the time of inspection not a single bunch of mistletoe was alive. Large and small bunches alike were dead. The mistletoe had evidently been dead for more than a year since all of the small aerial branches had fallen off leaving broken stubs some two or three inches long attached to the living juniper branches. An examination of the subcortical portions of the mistletoe showed that they were also dead.

The writer is now making a more intensive study of the mistletoe on this area with a view to determining the exact cause of its death. It is hoped that through this investigation some practical method may

be found of ridding ornamental trees of mistletoe infestation without serious injury to the trees.—W. H. LONG.

The October number of Phytopathology was issued December 5, 1912.